Patent
Attorney's Docket No. 028723-005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Forssen	)	Interference No. 103,469
	)	
	)	
v.	)	
	)	
Mehlhorn	)	Administrative Patent Judge:
	)	Ronald H. Smith
	)	

SECOND DECLARATION OF DAVID S. CAFISO, Ph.D.

BOX INTERFERENCE

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, David S. Cafiso, declare as follows:

1. My credentials are as set forth in my declaration dated February 27, 1995, and in my Curriculum Vitae, which was attached as Mehlhorn Exhibit 1.

2. I am familiar with what the state of the art was for methods for loading liposomes prior to September 17, 1985, which I understand to be the date on which application Serial No. 776,826 (the grandparent application to Mehlhorn, U.S. Patent Application Serial No. 547,382 ("Mehlhorn"), which is in interference) was filed. I am also familiar with what the level of skill in the art was prior to September 17, 1985. As such, I believe myself to be and to have

been a person of at least ordinary skill in the art relating to methods of loading liposomes prior to September 17, 1985.

3. I have read and reviewed the Preliminary Motion for Judgment filed by Forssen under 37 C.F.R. §1.633(a), including the Appendices and Exhibits attached thereto. Based upon my knowledge of the skill in the art at the time the Mehlhorn application was filed, and for the reasons given as follows, I do not agree with the assertion by Forssen that the cited art discloses or even suggests Mehlhorn's claimed invention.

a. I have reviewed both the specification and all of the claims (Claims 27-50) of Mehlhorn, which have an effective filing date of September 17, 1985.

b. As stated in my previous Declaration, I continue to believe that Mehlhorn Claims 27-50 are not disclosed or suggested by Nichols and Deamer. I reviewed Nichols and Deamer, "Catecholamine Uptake and Concentration by Liposomes Maintaining pH Gradients," *Biochimica et Biophysica Acta* 455:269-271 (1976) ("Nichols and Deamer"). Nichols and Deamer tested the possibility that catecholamines can be concentrated into liposomes using pH gradients. Catecholamines were accumulated using a pH gradient of 3.0. When the gradients were destroyed (e.g., by ammonium chloride additions) the accumulated catecholamines were released,

demonstrating that the uptake was reversible and dependent upon pH gradients. Nichols and Deamer thus postulated a "potential role of pH gradients in the uptake and concentration of catecholamines by sub-cellular storage sites" (p. 271, last paragraph).

Nichols and Deamer thus discloses the physical chemistry involved in using a pH gradient to load catecholamines into a liposome. However, Nichols and Deamer does not teach that the loaded drug composition can be accumulated and thus entrapped in the liposome. Nor was the application of this physical chemistry to drug-entrapped liposomes appreciated by those skilled in the art prior to September 1985.

Thus, there is no teaching or suggestion in Nichols and Deamer that a stable entrapped drug composition could be obtained as claimed by Mehlhorn. Nor would it have been obvious to one of ordinary skill in the art that a pH gradient could be used to prepare stable entrapped drug compositions as claimed by Mehlhorn. Therefore, Nichols and Deamer fails to disclose or suggest the invention as claimed by Mehlhorn in Claims 27-50.

c. I further believe that Cramer and Prestegard fails to disclose or suggest the invention recited in Mehlhorn Claims 27-50. I have also reviewed Cramer and Prestegard, "NMR studies of pH-Induced Transport of Carboxylic Acids Across Phospholipid Vesicle Membranes," *Biochemical and Biophysical Research Communications* 75(2):295-301 (1977) ("Cramer and Prestegard"). Cramer and

Prestegard demonstrated that transmembrane pH gradients across single-bilayer vesicle membranes effect the transport and concentration of carboxylic acids. The results of their studies indicated "that this transport occurs *via* selective permeation of the membrane by the protonated (uncharged) form of the acid" (page 295, Summary). Cramer and Prestegard tested the hypothesis that since the neutral form of a molecule, such as a carboxylic acid, is more lipid soluble and hence more permeable than its ionic counterparts, lowering the pH of a carboxylic acid solution, which is external to a closed vesicular membrane, should concentrate the acid in the interior volume of the vesicle.

More specifically, Cramer and Prestegard showed that lowering the outside pH will drive the transport of external fumaric acid into the interior of the vesicles (page 297, last paragraph). Lowering the pH still further (pH 4.7 vs. pH 5.5) resulted in a rapid and greater accumulation of internal fumaric acid, followed by a slow simultaneous leakage of both fumaric and maleic acid, which was also present in the liposome (page 298, last paragraph). The article further predicted, based on passive diffusion models, that following a pH perturbation, an equilibrium condition should be reached where the internal and external acid activities are equal. The experimental data showed the values to be equivalent and Cramer and Prestegard stated that this is evidence for the coupling of proton and carboxylate transport *via* the selective transport of the fully protonated acid form.

Cramer and Prestegard, like Nichols and Deamer, discloses the physical chemistry involved in using a pH gradient to load certain simple ionizable molecules into a liposome. However, there is no recognition that the physical chemistry involved in

using a pH gradient to load these simple ionizable molecules could also be applied to the accumulation and thus entrapment of a drug composition in the liposome. Nor was the application of this physical chemistry to drug-entrapped liposomes appreciated by those skilled in the art prior to September 1985.

There is no teaching or suggestion in Cramer and Prestegard that a stable entrapped drug composition could be obtained as claimed by Mehlhorn. Nor would it have been obvious to one of ordinary skill in the art that a pH gradient could be used to prepare stable entrapped drug compositions as claimed by Mehlhorn. Therefore, Cramer and Prestegard fails to disclose or suggest the invention as claimed by Mehlhorn in Claims 27-50.

d. It is also my opinion that Fendler neither discloses nor suggests Claims 27-50 of Mehlhorn. I have also reviewed Fendler, "Optimizing Drug Entrapment in Liposomes. Chemical and Biophysical Considerations," *Liposomes in Biological Systems*, Gregoriadis and Allison, Eds., pp. 87-100 (1980) ("Fendler"). In contradistinction to Nichols and Deamer and Cramer and Prestegard, Fendler is related to the use of liposomes as drug carriers. Fendler, which was published after both Nichols and Deamer and Cramer and Prestegard, in fact states as follows:

> Information, even at the supramolecular level, on factors affecting drug entrapment and retainment in liposomes as well as that on their fate *in vivo* is meager.

Fendler relates to the use of solubility to improve drug entrapment. For example, Fendler discloses that "[c]hanging the solubility of a drug by chemical modification is a relatively convenient way to ensure greater entrapment" (page 96, first paragraph). More specifically, Fendler states as follows under the heading "4. ENHANCING DRUG ENTRAPMENT IN LIPOSOMES" (page 96):

> Enhanced aqueous solubility of drugs can be readily achieved by adjusting the pH of the solution to ionize available functional groups. It is quite possible to maintain pH gradients in excess of 2 units for a period of several hours or longer across liposomes. . . . Proton permeability across the lipid bilayer is dependent upon the buffer used to adjust the pH inside the liposome. Using borate or phosphate buffer, pH gradients can be maintained for longer than 24 hours. Proton permeability in the presence of sodium acetate buffer occurs, however, within minutes. These data provide means for the more effective entrapment of drugs. It should also be possible to preserve sensitive drugs in the carriers at pH values different from the surrounding *in vivo* media. Slow release of liposome-entrapped liposome is a conceivable application.
>
> Formation of more soluble prodrugs is also a promising method for enhanced drug entrapment in liposomes. Incorporation of 8-azaguanine and 6-mercaptopurine increased dramatically by the addition of chloranil (Tsujii *et al.*, 1976; Table 2). Apparently, the chloranil-drug charge transfer complex is more soluble in the liposome than the drug. Subsequent to entrapment, the charge transfer complex readily decomposes to its parent donor and acceptor.

The use of the pH gradient in Fendler is related to maintaining the drug in an ionized state rather than using the pH gradient as an energy source to drive the drug

accumulation as claimed by Mehlhorn. Fendler thus is unrelated to the use of pH gradients to efficiently load and entrap a drug composition in a liposome, as claimed by Mehlhorn. Contrary to the assertion in the Preliminary Motion under 37 C.F.R. §1.633(a) (page 11, second full paragraph), the Fendler article does not teach "the use of buffers to maintain pH gradients for extended periods of time, thereby further increasing drug loading efficiency." Instead, Fendler teaches that *solubility* of a drug can be enhanced by adjusting and maintaining the pH of the interior vesicle solution. In view of the above, Fendler fails to disclose or even suggest the invention of Mehlhorn as recited in Claims 27-50.

e. As further evidence that Fendler is not referring to drug loading utilizing the physical chemistry described by Nichols and Deamer, I note that the article makes no reference to Nichols and Deamer, Cramer and Prestegard or my own work, (e.g., Cafiso and Hubbell (1978) as cited in my previous Declaration). Thus, Fendler, who was of at least average skill in the art did not acknowledge and/or was unaware of the potential of the use of a pH gradient to maintain or entrap drug compositions in the manner disclosed by Mehlhorn.

f. It is my opinion that none of the Nichols and Deamer, Cramer and Prestegard and Fendler articles, either alone or in combination, discloses or suggests that a stable entrapped drug composition could be obtained as claimed by Mehlhorn. Nor would it have been obvious to one of ordinary skill in the art that a pH gradient could be

used to prepare stable entrapped drug compositions as claimed by Mehlhorn. Therefore, the references cited by Forssen, either alone or in combination, fail to disclose or suggest the invention as claimed by Mehlhorn in Claims 27-50.

g. As a person skilled in the art, I would not agree with the assertion in the Preliminary Motion under 37 C.F.R. §1.633(a) (page 21, "b. Powerful Objective Evidence Demonstrates that Nichols Was the First Disclosure Demonstrating pH Loading of Lipophilic Cationic Drugs"), which states as follows:

> The objective evidence indicates that Nichols is credited by those skilled in the art as the first to teach pH loading of liposomes with lipophilic cationic drugs.

As a person skilled in the art, it is my opinion that Nichols and Deamer shows that ionizable compounds can be accumulated or loaded into vesicles using a pH gradient. However, it is my opinion that the skilled artisan prior to September 17, 1985, would not have considered Nichols and Deamer to have taught that entrapped drug compositions could be obtained, as claimed by Mehlhorn.

h. I also understand that Forssen is alleging that Cullis and coworkers acknowledge that Nichols and Deamer were the first to disclose the invention of the Count. I do not agree with this conclusion. It is my opinion that the work of Cullis, which was published after September 17, 1985, applied the teachings of Nichols and Deamer and showed that a pH gradient could be used to obtain an entrapped drug composition. This opinion appears to also be appreciated in the articles cited by Forssen

in the Preliminary Motion under 37 C.F.R. §1.633(a) (page 22-23), i.e., Haran et al and Barenholz et al, which state that the concept of Nichols and Deamer was used by Cullis and co-workers, who demonstrated that liposomes loaded by such an approach when compared with the free drug have lowered toxicity and improved efficacy. Thus, while Nichols and Deamer showed the uptake of catecholamines into liposomes using pH gradients, those skilled in the art recognize the difference between this showing and the work of others, e.g., Cullis and co-workers, in using this principle of physical chemistry to entrap drug compositions in a liposome.

i. It is my further opinion that it was not accepted by those skilled in the art, including Cullis and co-workers, that Nichols and Deamer taught the entrapment of drug compositions using a pH gradient, as claimed by Mehlhorn. The Cullis group published prolifically from 1986 forward, regarding the entrapment of drug compositions using a pH gradient. *See,* for example, Mayer et al, "Techniques for Encapsulating Bioactive Agents Into Liposomes," *Chemistry and Physics of Lipids* 40:333-345 (1986); Mayer et al, "Uptake of Adriamycin into Large Unilamellar Vesicles in Response to a pH Gradient," *Biochimica et Biophysica Acta* 857:123-126 (1986); and Hope et al, U.S. Patent No. 5,204,112, which were discussed in my prior Declaration. It is my opinion that if Cullis and co-workers believed that the idea of using a pH gradient for entrapment of drug compositions was already taught by Nichols and Deamer, they would not have spent so much time, effort and money on the research published in these articles, among others. Also, if Nichols and Deamer were recognized by others skilled in the art as

teaching drug entrapment using liposomes, as claimed by Mehlhorn, the work of Cullis' group would not have been accepted for publication by the review boards of journals (which comprise people highly skilled in the art) such as *Chemistry and Physics of Lipids* and *Biochimica et Biophysica Acta.*

j. As a result of work carried out over the past 10 years, we now recognize that many drugs can be accumulated by a mechanism that utilizes the same physical chemistry described in early articles of Nichols and Deamer, Cramer and Prestegard and Cafiso and Hubbell. However, for those skilled in the art prior to 1985, it would not have been clear whether such a mechanism would work as a general mechanism to accumulate drugs. Drugs such as doxorubicin do contain simple amine functionalities like the catecholamines examined by Nichols and Deamer. However, drugs such as doxorubicin also contain additional structural features not present in catecholamines. Thus, prior to 1985, those skilled in the art, even if they recognized the utility of the physical chemistry described by Nichols and Deamer, would not have known whether the additional structural features present in drugs such as doxorubicin would render the use of this physical chemistry unworkable for drug loading and entrapment.

k. Based upon the above, it is my opinion that, prior to September 17, 1985, those skilled in the art did not recognize the use of a pH gradient for loading a liposome as a method for preparing a stable drug entrapped liposome. Therefore, it is my

opinion that the assertion by Forssen that the claims of Mehlhorn are disclosed or suggested by Nichols and Deamer, Cramer and Prestegard, and Fendler, either alone or in combination, is unfounded.

4. I have also read and reviewed the Preliminary Motions for Judgement filed by Forssen under 37 C.F.R. §1.633(c)(4), including the Appendices and Exhibits attached thereto. Based upon my knowledge of the skill in the art at the time the Forssen application was filed, and for the reasons given as follows, I do not agree with the assertion by Forssen that the use of pyranosidyl acids, e.g., lactobionic acid and galacturonic acid, would have been nonobvious to a person skilled in the art at the time the application was filed.

a. It is well recognized in the art that there were two criteria for selecting a buffer to use for preparing a drug-entrapped liposome composition using a pH gradient. The criteria were that (1) the buffer is biocompatible, and (2) the buffer is non-membrane permeable. There was a wide range of compounds which satisfy these two criteria; the ultimate selection of what buffer to use was often a matter of choice, affected by considerations such as cost and ready availability.

b. Sugars, such as the pyranosidyl acids recited in Claims 5, 6 and 25-27, were recognized by those skilled in the art as being (1) biocompatible and (2) non-membrane permeable. Thus, the use of pyranosidyl acids as buffers for preparing drug-entrapped liposome compositions was consistent with knowledge in the art at the time of

the Forssen patent. That such acids were effective for forming liposomal compositions would not have been surprising.

c. The Preliminary Motion states that there is no suggestion in the prior art or in the generic claims corresponding to the Count in the Interference that the selection of a particular acidic species will have any effect, particularly a significant positive effect, on the pH loading process or the products resulting from it. This statement is not true. As described above, buffers must be biocompatible and impermeable. It was well recognized in the art that acids are membrane impermeable to different degrees based on their structure and their pK_a, which is indicative of the ability of the hydrogen ion to dissociate from the acid. In particular, compounds having a structure comprising, for example, a carboxylic acid and a weak base or permanently ionizable group (e.g., a quaternary amine or ammonium), will be highly impermeable. Moreover, acids having a pK_a of less than 1 will be highly impermeable because they are highly ionized even at relatively low pHs. One skilled in the art would, therefore, have recognized that the particular acidic species used as a buffer can have a significant positive effect on the pH loading process or the products resulting therefrom.

d. The Preliminary Motion purports that Forssen found that doxorubicin could be loaded into liposomes with lactobionic and galacturonic acids, but not with acetic acid, and that when citric acid was used, the doxorubicin liposomes were more toxic than the free drug.

Compounds will cross the liposome membrane in the uncharged form, i.e., in the neutral state. If a compound readily exists in the uncharged form, a pH gradient will not be maintained. Acetic acid has a pK_a of about 4. Since the pK_a of the acid is not very low, one would expect the acetic acid to be present in the neutral state, to readily cross the liposome membrane and thus to not be useful for maintaining a pH gradient. The fact that acetates are recognized in the art as being poor buffers for maintaining a pH gradient is also indicated by Fendler. It is, therefore, not surprising that Forssen found acetic acid to not be useful for loading doxorubicin into liposomes.

Citric acid has three ionizable groups, with pK_as of 7, 4 and 3. Therefore, citric acid is not as likely as acetic acid to be present in the neutral state. However, since these pK_as are not very low, some citric acid may be present in the neutral state. Citric acid, as used in the Mehlhorn application, would, therefore, be expected to be superior in terms of liposome retention as compared to acetic acid. Similarly, lactobionic and galacturonic acids, by virtue of their structure and many hydroxyl groups, would have been expected to be retained to an even greater degree within the liposome.

Sugars, such as the pyranosidyl acids, were also known to be biocompatible. They were also known to be highly impermeable because of the many hydroxyl groups present on the molecule. A highly impermeable buffer would have been expected to possess properties different from those of other acids, such as acetic acid and citric acids. More specifically, it was not surprising that a pyranosidyl acid could be used as a buffer for loading a drug composition using a pH gradient and that it could possibly be more effective than acetic acid or citric acid.

e. In particular, I have reviewed the examples of the Forssen patent, which are asserted to demonstrate unexpected results for preparing a lipophilic drug containing liposomal composition using a monofunctional pyranosidyl acid to form the liposomes and load the drug into the liposomes. As a person of at least ordinary skill in the art, it is my opinion that this conclusion cannot be made based on the data set forth in the examples of Forssen.

Forssen only shows a comparison of doxorubicin entrapped liposomes, wherein the doxorubicin is entrapped in distearoyl phosphatidlycholine-cholesterol vesicles using lactobionic acid and calcium carbonate and wherein the doxorubicin is entrapped in distearoyl phosphatidlycholine-cholesterol vesicles using citric acid and calcium carbonate. Superior results in terms of toxicity is thus shown only for the specific combination of doxorubicin and lactobionic acid in comparison with the specific combination of doxorubicin and citric acid.

Based on this showing, it is my opinion that it cannot be concluded that all lipophilic drugs loaded into the liposome using a monofunctional pyranosidyl acid will be superior to such liposomes loaded using all other organic acids. First, Forssen has only shown the combination of one lipophilic drug with one monofunctional pyranosidyl acid. Second, Forssen has only compared this particular combination with one other organic acid, i.e., citric acid. By reference to Forssen, column 5, lines 43-61, other acids were known to be useful for loading ionizable molecules into liposomes. This is also evidenced by the prior art's use of other organic acids, for example, the Cullis group's use of amino acids such as glutamic acid (*see*, Mayer et al, *Biochimica et Biophysica Acta*,

and Hope et al, U.S. Patent No. 5,204,112). Based on this data, I cannot conclude whether the use of pyranosidyl acids is better, much less unexpectedly better, than the use of organic acids for loading all lipophilic drugs.

Moreover, Forssen does not even show beneficial results for galacturonic acid, the other pyranosidyl acid specifically mentioned in the patent. Example VIII, which shows the biological activity of doxorubicin vesicles, compares only lactobionic acid and citric acid, and reports that the studies are "still continuing." No comparison of galacturonic acid and citric acid has been evidenced by Forssen. Thus, it cannot be concluded that all pyranosidyl acids are even better than citric acid.

Further, with respect to the drug composition, Forssen only shows doxorubicin. Example VIII shows only that the maximum tolerated dose for doxorubicin liposomes loaded using lactobionic acid is higher than for those loaded using citric acid and that the maximum tolerated dose of the free drug doxorubicin is higher than for the citric acid loaded liposomes.

As can be seen in Example VI, however, the toxicity of daunorubicin liposomes loaded into liposomes using citric acid is lower than for the free drug and the antitumor activity for the liposomes is higher than for the free drug. Based upon this data, it can be concluded that citric acid is an acceptable buffer for loading antineoplastic drugs. In addition, the results obtained using citric acid for this antineoplastic drug are not compared with a similar liposome loaded using a pyranosidyl acid.

Therefore, as a person of at least ordinary skill in the art, I cannot conclude based on the showing made by Forssen whether any unexpected or even

beneficial results are obtained by using a monofunctional pyranosidyl acid to form a lipophilic drug containing liposome. The only showing of Forssen is that the one combination of doxorubicin and lactobionic acid appears to have lower toxicity than the combination of doxorubicin and citric acid when used to form drug containing liposomes. Based on the data presented, however, I cannot conclude whether or not this is a result particular to that one combination. Sufficient data for reaching any conclusion regarding the results obtained in Example VIII, other than that the particular combination appears to lower toxicity, has not been presented.

f. Based upon the above, it is my opinion that the use of pyranosidyl acids as buffers for preparing drug-entrapped liposomes using a pH gradient would have been obvious to a person of ordinary skill in the art based upon the art-recognized impermeability and biocompatability of such compounds. Moreover, Forssen has not shown that the general use of pyranosidyl acids to form lipophilic drug containing liposomes results in unexpected and beneficial results.

5. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issuing thereon.

4-3-95
Date

David S. Cafiso, Ph.D.